

# Hereditary Deficiency of the Fifth Component of Complement in Man

## I. CLINICAL, IMMUNOCHEMICAL, AND FAMILY STUDIES

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**ABSTRACT** The first recognized human kindred with hereditary deficiency of the fifth component of complement (C5) is described. The proband, a 20-year-old black female with systemic lupus erythematosus since age 11, lacked serum hemolytic complement activity, even during remission. C5 was undetectable in her serum by both immunodiffusion and hemolytic assays. Other complement components were normal during remission of lupus, but C1, C4, C2, and C3 levels fell during exacerbations.

A younger half-sister, who had no underlying disease, was also found to lack immunochemically detectable C5. By hemolytic assay, she exhibited 1–2% of the normal serum C5 level and normal concentrations of other complement components. C5 levels of other family members were either normal or approximately half-normal, consistent with autosomal codominant inheritance of the gene determining C5 deficiency.

Normal hemolytic titers were restored to both homozygous C5-deficient (C5D) sera by addition of highly purified human C5. In specific C5 titrations, however, it was noted that when limited amounts of C5 were assayed in the presence of low dilutions of either C5D serum, curving rather than linear dose-response plots were consistently obtained, suggesting some inhibitory effect. Further studies suggested that low dilutions of C5D serum contain a factor (or factors) interfering at some step in the hemolytic assay of C5, rather than a true C5 inhibitor or inactivator.

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Of clinical interest are (a) the documentation of membranous glomerulonephritis, vasculitis, and arthritis in an individual lacking C5 (and its biologic functions), and (b) a remarkable propensity to bacterial infections in the proband, even during periods of low-dose or alternate-day corticosteroid therapy. Other observations indicate that the C5D state is compatible with normal coagulation function and the capacity to mount a neutrophilic leukocytosis during pyogenic infection.

## INTRODUCTION

In the last decade isolation and functional characterization of most of the proteins of the complement (C)<sup>1</sup> system has permitted increasing recognition of humans and experimental animals with deficiency of single components of this system. In man, individuals or families with deficiencies of C1q, C1r, C1s, C4, C2, C3, C6, C7, C8, C1 esterase inhibitor and C3b inactivator have been reported (1–14). These hereditary C-deficiency states have provided unique insights concerning the biological functions of this important effector system. Some of these C-deficient humans have had increased susceptibility to infection (2–4, 11, 12); others have had associated rheumatic, renal, or autoimmune disorders (2, 3, 6–10, 13); still other C-deficient individuals have been healthy (1, 5, 10).

We report here the first known cases of hereditary C5 deficiency in man, with analysis of the immunochemical basis of the defect, its mode of inheritance, and related clinical observations.

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<sup>1</sup>Abbreviations used in this paper: C, complement; CH<sub>50</sub>, hemolytic complement; C5D, C6D, etc., C5-deficient, C6-deficient, etc.; SLE, systemic lupus erythematosus.

## Clinical summary

**Case 1.** The proband, L. H., is a 20-year-old black female. During early childhood she experienced relatively frequent skin, ear, upper respiratory, and vaginal infections not requiring hospitalization. Measles and mumps infections were uneventful. She first presented to the University of Rochester Medical Center in September 1965 at age 11, with a recent onset of fatigue, weight loss, sore mouth and throat, Raynaud's phenomenon, and joint pains. Initial evaluation revealed blood pressure of 138/96, multiple swollen tender joints without definite effusions, cold-blanching extremities, mild Coombs' negative anemia, and leukocytosis. Her urine contained erythrocytes, leukocytes, granular and erythrocyte casts, and trace to 1+ protein. Erythrocyte sedimentation rate was 126 (Westergren method). Lupus erythematosus cell preparations and antinuclear antibody determinations were strongly positive. A latex fixation test for rheumatoid factor was negative. Serum protein electrophoresis revealed decreased albumin and diffusely increased  $\gamma$ -globulin. IgG, IgA, and IgM were elevated. Antistreptolysin O titer has been consistently elevated ( $\sim 2,000$  Todd units) despite absence of clinical or bacteriologic evidence for streptococcal infection on many occasions. Hemolytic complement ( $CH_{50}$ ) activity was undetectable and has remained so throughout her course. In 1971, the patient's cutaneous delayed hypersensitivity was tested with seven standard antigens, two of which (monilia and mosquito) gave positive responses. Serum urea nitrogen, creatinine, and creatinine clearance were normal. Hemoglobin electrophoresis revealed 55% hemoglobin A and 45% hemoglobin S.

A renal biopsy in 1965 showed tubular casts, focal glomerular basement membrane thickening, and multiple foci of lymphocytes in the interstitium. Electron microscopy, performed by Dr. Bernard Panner (Department of Pathology), revealed subepithelial electron-dense deposits and endothelial intracytoplasmic "tubular" inclusions. In 1970 a repeat renal biopsy showed moderate progression to a more diffuse membranous glomerulonephritis with mild hypercellularity of glomeruli. Immunofluorescent study of this specimen revealed granular deposition of IgG, IgA, and IgM in the glomerular basement membrane, with negative staining for C3, fibrinogen, and albumin. Coagulation tests before the 1970 biopsy revealed normal bleeding time (Ivy), partial thromboplastin time, prothrombin time, and platelet factor III activity. In 1972 a test for cryoglobulins revealed 1% "cryocrit." In 1974 a skin biopsy showed marked dermal edema with focal necrosis of collagen, liquefactive degeneration of basal cell layer, chronic perivascularitis, and immunofluorescent deposits of IgG, IgM, and C3 along the dermal-epidermal junction.

A diagnosis of systemic lupus erythematosus (SLE) with nephritis was made on her first admission, and she was begun on prednisone. Over the years her management has required continued prednisone therapy, in doses varying from 80 mg daily to 15 mg every other day. The manifestations of SLE responded to this therapy, but her subsequent course has been characterized by intermittent lupus activity and very frequent infections. Flareups of SLE, which commonly accompanied attempts to reduce prednisone dosage, usually consisted of fever, arthritis or arthralgia, neutropenia, cutaneous vasculitis, oral and vaginal ulcerations (frequently complicated by monilial infection), and mild proteinuria, with good preservation of renal function. Such exacerbations, which have always responded to increases or re-institution of prednisone therapy, were typically accompanied by reductions in serum C3 and C4 to about half-normal levels and elevations of serum anti-DNA (both "native" and heat-denatured) antibodies whenever these were measured. During periods of remission, these laboratory parameters frequently returned to normal, although her  $CH_{50}$  remained undetectable.

The patient's numerous infections (Table I) have included a major problem of recurrent, indolent, infected cutaneous ulcers and subcutaneous abscesses with chronically draining sinus tracts, particularly on the extremities and scalp. These occurred even during periods of low-dose or alternate-day corticosteroid therapy. Exudates from these areas have shown numerous neutrophils on several occasions, but at other times cells were sparse. Typical organisms isolated include staphylococcus sp., proteus sp., pseudomonas sp., enterobacter sp., and *Streptococcus pyogenes*.

The patient has also been troubled by chronic oral and vaginal moniliasis. An episode of herpes zoster began in November 1971 while the patient was taking prednisone, 40 mg/day. This eruption persisted until March 1972 (while she was taking mainly alternate-day prednisone, 40-80 mg), but did not disseminate. In 1972 she had a life-threatening infection beginning with bilateral axillary abscesses with mixed gram-negative and gram-positive flora, and culminating in enterococcal sepsis, meningitis with delirium and aphasia, and possible subacute bacterial endocarditis. These responded to intensive antibacterial therapy, and she regained full neurological function. That illness was further complicated by acute renal papillary necrosis with gross hematuria, and transient glucose intolerance requiring insulin therapy for several months. In 1973 and 1974 her urine and blood sugars were normal with no further diabetic therapy, but in the past year, development of marked exogenous obesity has been accompanied by insulin-requiring glucose intolerance. Persistent shedding of cytomegalovirus has also been found in her urine but is of uncertain

TABLE I  
Clinical Summary: Proband

Age	Diagnosis
yr	
3-11	Frequent ear, skin, and vaginal infections; measles, mumps uneventful.
11	Onset of classic SLE with polyarthritis, Raynaud's phenomenon, positive lupus erythematosus cell test and antinuclear antibody, hyper- $\gamma$ -globulinemia, undetectable hemolytic C. Renal biopsy: focal nephritis.
12-13	Stable.
14-15	Axillary abscesses; otitis media and externa.
16	Lupus flare-up with cutaneous vasculitis. Repeat renal biopsy: diffuse membranous glomerulonephritis.
16-18	Alopecia; oral and vaginal moniliasis; prolonged but localized herpes zoster.
18	Axillary abscesses; enterococcal septicemia with meningitis. Transient glucose intolerance.
18-20	Cutaneous vasculitis with ulceration; multiple subcutaneous abscesses (pseudomonas, staphylococcus); nephritis, inactive.
19	Femoral thrombophlebitis with pulmonary embolus.
20	SLE controlled on alternate-day prednisone; exogenous obesity; diabetes mellitus.

clinical significance. Although her peripheral blood leukocytes usually tended to be low (1,500-5,000), some but not all episodes of pyogenic infection were accompanied by brisk neutrophilic leukocytosis to 16-18,000 with marked left shift.

In April 1974, during a period of immobilization for therapy of leg ulcers, the patient developed deep thrombophlebitis in the left calf with pulmonary embolization. This responded to anticoagulation therapy, and she has subsequently been maintained on warfarin. The patient has never received blood or plasma transfusions and has never been pregnant.

Family history includes diabetes mellitus and carcinoma of the biliary tract in her mother. Her father died suddenly at age 31 after an episode which was said to be "either a heart attack or blood clot in the lung."

*Case 2.* The half-sister of the proband, D. H., is a 10-year-old black girl who was born during the mother's second marriage. She has been in good health except for frequent upper respiratory infections during early childhood leading to chronic anterior cervical lymphadenopathy, episodes of pneumococcal pneumonia at the ages of 18 mo and 5 yr, and one episode of nonspecific bacterial vaginitis at age 8. More recently, her health has been excellent. Physical examination in June 1974 was normal except for nontender cervical adenopathy, and

was entirely normal in May 1975. Laboratory evaluation (1974 and 1975) has revealed no evidence of SLE, including negative tests for antinuclear antibody, anti-DNA antibodies, and rheumatoid factor, as well as normal serum immunoglobulin levels. Her CH<sub>50</sub> titer is 45 U/ml (normal 80-160). Normal values were obtained in standard coagulation studies, including whole blood-clotting times in glass and plastic at 25°C, prothrombin time, activated partial thromboplastin time, clot retraction, kaolin-induced platelet factor III release, and clot lysis time (performed by Dr. Robert T. Breckenridge, Rochester General Hospital, Rochester, N. Y.).

Other siblings (or half-siblings) of cases 1 and 2 and their guardian were interviewed briefly; symptoms suggestive of infectious, rheumatic, or other chronic diseases were denied.

## METHODS

*Human sera.* Freshly obtained sera were stored at -70°C in small aliquots. Unless specifically stated to the contrary, sera of the C5D proband chosen for study were from periods of clinical remission.

*Specific antisera.* Specific antisera to human IgM, IgG, and IgA were prepared in rabbits as previously described (15), as was rabbit antiserum to human C4 (16). Rabbit antiserum to human C3 was the gift of Dr. John T. Boyer, University of Arizona School of Medicine, Tucson, Ariz. Goat antiserum to human C5 was the gift of Dr. Ulf Nilsson, University of Pennsylvania School of Medicine, Philadelphia, Pa. Antisera to human properdin factor B and C5 were supplied by Dr. Chester A. Alper, Children's Hospital Medical Center, Boston, Mass. Rabbit antiserum to erythrocyte hemolysin was purchased from Baltimore Biological Laboratories (Baltimore, Md.). Additional rabbit antisera to human C5 (17) were prepared in this laboratory.

*Complement reagents and assays.* Plates for measuring human C3, C4, and C5 by radial immunodiffusion were purchased from the Hyland Division, Travenol Laboratories (Costa Mesa, Calif.; C3) and Meloy Laboratories, Inc. (Springfield, Va.; C4 and C5). Normal ranges were established with sera from 20 healthy donors.

CH<sub>50</sub> titers were determined as previously reported (18). Individual, functionally pure guinea pig and human complement components C2 and C3-9 were obtained from Cordis Laboratories (Miami, Fla.). Functionally pure guinea pig C1 was prepared according to the method of Nelson and associates (19). Highly purified human C5 was initially the generous gift of Dr. Ulf Nilsson, and for later experiments was prepared in this laboratory by the method of Nilsson et al. (17). This preparation showed a single (C5) arc in immunoelectrophoresis against polyvalent horse antihuman serum and produced one main band and one adjoining faint band in polyacrylamide gel electrophoresis, as described by Nilsson et al. (17). Functional hemolytic assays of C1, C4, and C2 were performed according to Rapp and Borsos (20) except that reaction volumes were reduced by half. Guinea pig serum diluted in 0.01 M EDTA was used as the source of C3-9 and cellular intermediates were made using C1<sub>gp</sub>, C4<sub>hu</sub>, and <sup>oxy</sup>C2<sub>hu</sub> (21). Titrations of late components, C3-9, were performed according to the methods of Nelson et al. (19) except that <sup>oxy</sup>C2<sub>hu</sub> was used instead of C2<sub>gp</sub>, and the

reaction volumes were reduced. The assay for C5 is representative of other late component assays, and was performed as follows: 0.2 ml of a given test dilution of the unknown sample in pH 7.2 dextrose-Veronal-buffered saline containing  $1.5 \times 10^{-4}$  M  $\text{Ca}^{++}$ ,  $5 \times 10^{-4}$  M  $\text{Mg}^{++}$ , and 0.1% gelatin (DGVVB<sup>++</sup>) (20) was added to 0.2 ml of DGVVB<sup>++</sup>. To this was added 0.2 ml of a mixture containing 50 U/ml each of C3<sub>hu</sub>, C6<sub>hu</sub> and C7<sub>hu</sub>, and <sup>oxy</sup>C2<sub>hu</sub> (25 U/ml before oxidation with I<sub>2</sub> reagent) (21), as well as 0.2 ml of EAC1<sub>gp</sub>4<sub>hu</sub> ( $1 \times 10^8$  cells/ml, T<sub>max</sub> = 2–5 min). After incubation of this mixture at 30°C for 30 min, 0.2 ml of a mixture containing 50 U/ml each of C8<sub>gp</sub> and C9<sub>gp</sub> was added, and the tubes were then incubated at 37°C for 60 min. 2 ml of ice-cold saline was added to stop the reaction, and the degree of hemolysis was determined spectrophotometrically (412 nm). The percent lysis, corrected for reagent lysis, was plotted according to the Poisson distribution and the one-hit theory of complement lysis (22). The titer of a serum was taken to be the reciprocal of the dilution giving an average of one hemolytic site per cell ( $Z=1$ ). A standard normal serum pool representing 30 donors was titrated in each day's experiment, and all titers were corrected to correspond to a C5 value of 250,000 U/ml for the normal pool. Critical experiments comparing various sera were always run on the same day with a single batch of reagents. In assays of C3, C6, or C7 the same procedure was followed but with C5<sub>hu</sub> (50 U/ml) substituted for the component under study. Assays of C8 and C9 employed a cellular intermediate EAC1-7 prepared by treating EAC1<sub>gp</sub> with a reagent containing C4, C2, C3, C5, C6, and C7<sub>hu</sub> (C4-7 reagent, Cordis Laboratories, Miami, Fla.). These cells were incubated with test dilutions and excess of either C9<sub>gp</sub> or C8<sub>hu</sub> for 60 min at 37°C. Results were plotted as above.

*Tests for activation of alternative complement pathway.* Inulin activation (23) of normal and C5D sera was tested by incubating 0.12 ml of a 50-mg/ml saline suspension of inulin (Fisher Scientific Co., Pittsburgh, Pa.) with 0.48 ml of serum at 37°C. At 15, 30, 60, and 120 min, aliquots were removed and centrifuged at 900 *g* for 10 min at 4°C, and the supernatant solutions were subjected to immunoelectrophoresis in 1% agar in 0.02 M barbital buffer, pH 8.4, containing 0.01 M disodium EDTA. Antisera monospecific for C3 and properdin factor B were used, and the extent of conversion from C3 to the more anodal C3c and of factor B to its  $\alpha$ - and  $\gamma$ -globulin conversion products was estimated visually.

## RESULTS

*Characterization of the complement deficiency.* With the recognition that CH<sub>50</sub> activity in the proband's (L. H.) serum remained consistently undetectable despite remission of SLE and return of immunochemically measured C3 and C4 levels to normal, a defect in one of the late-acting complement components was suspected. This was confirmed in a screening assay in which EAC142 cells showed no lysis when tested against her serum in 0.01 M EDTA as the source of late C components. Mixtures of L. H. serum with C2D or C6D human serum yielded normal CH<sub>50</sub> titers. Detailed analysis of the serum C components of L. H. and her younger half-sister, D. H., is shown in Table II. It is apparent that the complement defect is limited to C5. C5 was also undetectable in EDTA plasma. C1, C4, C2,

TABLE II  
Complement Components of C5D Sera

Assay	C5D proband			Normal sera	
	SLE active	SLE inactive	C5D sibling	Pool*	Individual†
Hemolytic					
C1	61,300	79,100	81,800	90,000	75,000±12,500 (11)§
C4	29,300	101,900	72,206	121,500	117,200±45,200 (13)
C2	3,520	6,970	6,970	8,500	9,995± 3,100 (8)
C3	8,448	21,360	25,560	27,000	31,900± 8,700 (8)
C5	0	0	3,300	250,000	264,300±82,200 (23)
C6	234,523	251,771	194,526	140,000	121,600±22,800 (4)
C7	158,000	158,000	130,640	140,000	119,400± 6,780 (3)
C8	155,778	193,368	168,238	145,000	120,800±35,000 (7)
C9	83,660	99,200	64,000	80,000	
Immunodiffusion					
				Normal range	
C4	12.5	24	33	20–40 mg/100 ml	
C3	39	91	118	70–220 mg/100 ml	
C5	0	0	0	90–130 µg/ml	
C1 inhibitor	31.2	33	16.8	12–32 mg/100 ml	
Properdin factor B-Normal by immunoelectrophoresis					

\* Pool of 30 normal sera assayed concurrently with C5D sera.

† M ± SD representing normal values for this laboratory.

§ Numbers in parentheses indicate numbers of normal sera tested.

and C3 levels were low in L. H. serum during exacerbation of SLE, but were normal during remission. As expected, there was no depression of C components reacting after C5 during active SLE (Table II). With five goat or rabbit antisera to human C5, no C5 protein was detectable in either D. H. or L. H. serum by Ouchterlony double diffusion, radial immunodiffusion (Table II), or counter-immunoelectrophoresis. CH<sub>50</sub> titers in both C5D sera could be restored to normal by addition of highly purified human C5 (Table III).

TABLE III  
Reconstitution of C5D Serum with Highly Purified Human C5

Serum	Calculated C5 added*	Measured C5 protein†	Measured hemolytic C5	CH <sub>50</sub> titer
	µg/ml	µg/ml	U/ml	U/ml
L. H.	0	<2	0	0
	73	ND§	170,300	120
	165	145	315,400	145
D. H.	0	<2	~3,000	45
	73	ND	167,000	122
	175	155	370,200	136
Normal	0	140	315,400	148
	165	ND	740,500	ND

\* Amounts of C5 added calculated from concentration of purified C5. A dilution factor accounts for the difference between amount added and measured concentration.

† Radial immunodiffusion performed on C5:serum mixtures.

§ Not determined.

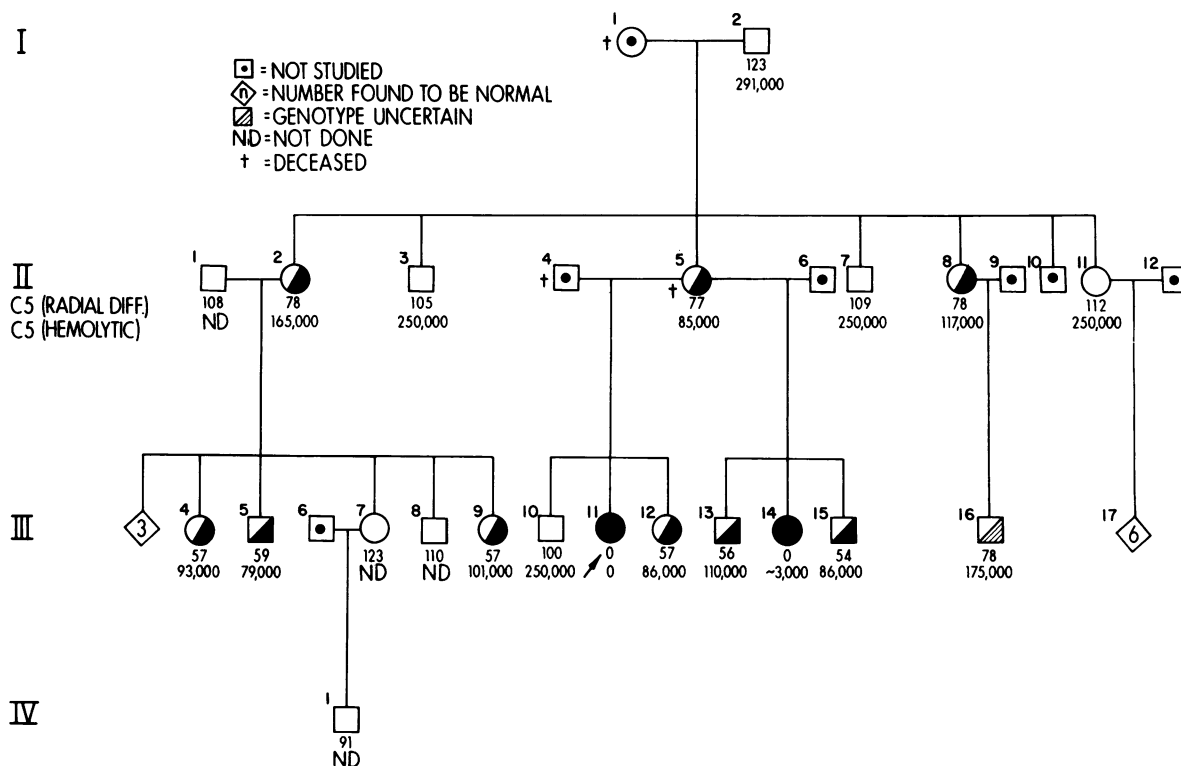


FIGURE 1 Family pedigree showing C5 values by radial immunodiffusion (normal range 90–130  $\mu\text{g/ml}$ ) and hemolytic assay (mean  $\pm$  SD for 15 normals:  $269,500 \pm 92,700$  U/ml). (●), proposed homozygotes; (◐), proposed heterozygotes; (□, ○), normals.

As a test of properdin (alternative) pathway function (23), the capacity of inulin to cause immunoelectrophoretic conversion of C3 and factor B in both C5D sera was examined in kinetic fashion. The changes ob-

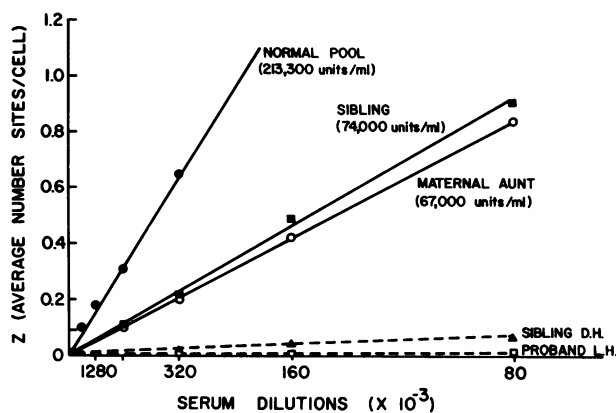


FIGURE 2 Hemolytic C5 titrations of sera from both C5D homozygotes, two proposed heterozygotes, and a pool of 30 normal sera. Z (average number of sites per cell) is calculated according to the Poisson distribution, as  $-\ln(1 - \text{1\% hemolysis})$  of a suspension containing  $1 \times 10^8$  sheep erythrocytes/ml. The C5 titer is taken as the reciprocal of the dilution producing an average of one site/cell.

served in D. H. serum were entirely comparable to those in the concurrently run control serum. Both the rate and extent of conversion of C3 in certain bleeding dates of the proband's (L. H.) serum appeared to be somewhat reduced, but in other samples were normal. This did not appear to correlate well either with the initial level of C3 in the serum or with the rate of conversion of factor B, which was usually comparable to the normal control.

**Family studies.** The pedigree is shown in Fig. 1. C5 levels measured by radial immunodiffusion and hemolytic assay are listed under the symbols. Both normal individuals and apparent heterozygotes, having approximately half-normal C5 levels, were found among the proband's siblings and first cousins in generation III, and among her maternal aunts in generation II. CH<sub>50</sub> titers on the apparent heterozygotes were normal (not shown) or, in one instance (II-8), elevated, indicating that C5D heterozygotes would not be detected by this screening method. The proband (L. H.) and her half-sister (D. H.) appear to be homozygous for the defect.

**Inhibitor studies.** When the usual serum dilutions were used in the hemolytic assay for C5, L. H. serum produced no detectable hemolysis, and the serum of D. H. gave minimal hemolysis, apparently in a stoichio-

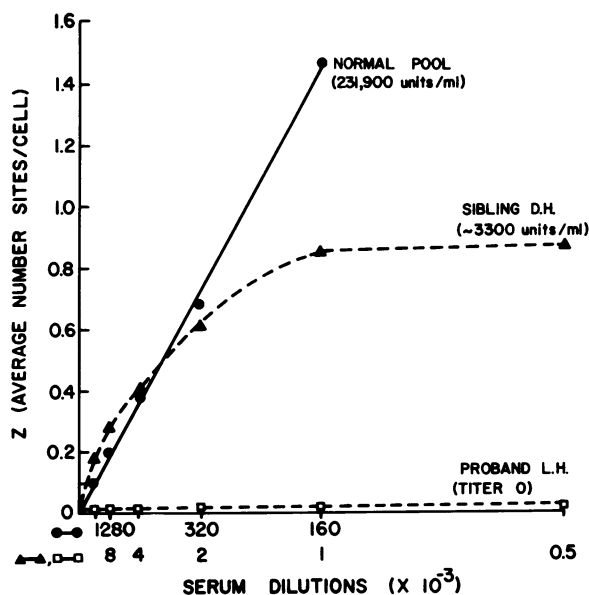


FIGURE 3 Hemolytic C5 titrations of a pool of 30 normal sera at the standard serum dilutions, and homozygous C5D sera at lower dilutions. Calculations as for Fig. 2. Approximate titer for D. H. serum was derived by extrapolating the first part of the curve to  $Z = 1$ .

metric fashion (Fig. 2). Concurrently tested sera from apparent heterozygotes also exhibited stoichiometric C5 titrations, as illustrated by two sera in Fig. 2. However, when lower dilutions of D. H. serum (1/500–1/16,000) were tested, the titration curve was consistently concave toward the abscissa (Fig. 3), suggesting the presence of an inhibitor. The hemolytic C5 value for D. H. serum listed in Table II was estimated by extrapolation from the earliest (and straightest) portion of the curve in Fig. 3. L. H. serum gave no detectable hemolysis in any dilution, even at 1/25 (not shown). However, when a limited quantity of C5 was added to L. H. serum, and the C5-serum mixture diluted 1/500–1/16,000 times, a similarly curved plot was obtained.

To study this possible inhibitory effect further, a constant amount<sup>a</sup> of purified human C5 (Cordis) was added to a series of tubes containing 0.1 ml of D. H. or L. H. serum in doubling dilutions from 1/800 to 1/51,200 in an ice bath. These mixtures were immediately assayed for C5 hemolytic activity. The average number of hemolytic sites per cell ( $Z$ ) was determined for each mixture, corrected for the number of sites produced by D. H. serum alone (L. H. serum produced no detectable sites), and calculated as percent inhibition of the sites formed by purified C5 alone. The degree of inhibition was similar for both C5D sera, and was dose-dependent, ranging

<sup>a</sup> This quantity of C5, in the absence of C5D serum, gave a  $Z$  value of 1.76 in the C5 assay (see Methods).

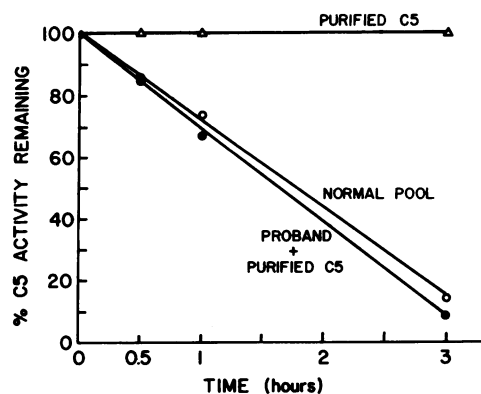


FIGURE 4 C5 decay in normal serum (●) and reconstituted C5D serum (○) during incubation at 37°C. Values are given as the percent of the starting titer.

from approximately 90% inhibition at a 1/800 serum dilution to 10% at a 1/51,200 dilution.

A constant dilution of each C5D serum (1/6,000) was then mixed with varying concentrations of functionally pure human C5 (Cordis) and immediately assayed for C5. The titer of the C5 preparation mixed with buffer alone in the initial step was 2,900 U/ml, and the titer of the C5 mixed with either C5D serum was 1,510 U/ml, a 48% reduction. It is noteworthy that the latter titrations were stoichiometric (see Discussion). Entirely comparable results were obtained when serially diluted human serum was employed as C5 source. This inhibitory effect of C5D serum was abolished by heating at 56°C for 30 min.

In another experiment, equal volumes of D. H. and normal human serum were mixed, incubated at 37°C for 15 min, and then serially diluted as usual for C5 titration. The mixture had a C5 titer of 134,200, and the normal serum, similarly incubated, had a titer of 266,700 U/ml. Thus, no inhibition of C5 beyond that accounted for by dilution was observed. Similarly, both L. H. and D. H. serum could be readily reconstituted to normal C5 titers by the addition of highly purified C5 in the expected amounts (Table III). At the high serum dilutions employed for these C5 assays, both titrations were linear.

Finally, L. H. serum was reconstituted with highly purified human C5 to a titer of 432,000 U/ml, and the rate of degradation of C5 in this mixture was compared with that in the normal serum pool mixed with buffer alone (starting titer 222,000 U/ml). At 37°C, the rate of C5 decay in the two mixtures was very comparable over a 3-h period (Fig. 4). C5 in buffer alone was stable under these conditions.

## DISCUSSION

The development of SLE in a young black female led ultimately to the recognition of a previously unreported C-deficiency state in man, hereditary C5 deficiency. The proband, L. H., had no detectable C5 activity in her

serum by hemolytic assay or C5 protein by various immunodiffusion methods. The proband's younger half-sister, D. H., also lacked measurable C5 protein, but in the more sensitive hemolytic assay exhibited approximately 1–2% of normal C5 activity. The differences in C5 levels between these two half-siblings could reflect differing paternal inheritance (Fig. 1) or possibly ongoing C5 consumption at a low level in the older girl with SLE, despite other evidences of remission at the time of study. The mother, several other siblings, maternal aunts, and first cousins, whom we propose to be heterozygotes, displayed hemolytic C5 levels 34–65% of the normal mean. The pedigree (Fig. 1), although incomplete in that the fathers (II-4 and II-6) of the two proposed homozygotes (III-11 and III-14) were unavailable, is nevertheless consistent with autosomal codominant inheritance of the putative C5D gene, in keeping with most other genetic deficiencies of C proteins (1–9). Setting aside questions of true paternity, the pedigree suggests that both fathers are heterozygotes, seemingly an extraordinary coincidence. This interpretation is made less remote perhaps by the fact that both men belonged to a migrant farm-laborer community which came to Rochester from west-central Florida. Possibly the C5D gene, which would not be detected in its heterozygous state by CH<sub>50</sub> titrations (see above), is less rare than presently suspected. The alternative possibility that II-6 (Fig. 1) was the actual father of both homozygotes cannot be excluded since he is unavailable for genetic typing.

Although addition of purified human C5 to either homozygous C5D serum restored normal CH<sub>50</sub> and C5 titers (Table III), the question of a C5 inhibitor (or inactivator) was explored at some length because of the nonlinear C5 titrations consistently observed with low dilutions of D. H. serum (Fig. 3) and because of the inhibitory effect of low dilutions of both C5D sera on C5 titers when a limiting quantity of C5 had been added. The observation that a constant amount of either C5D serum had the same inhibitory effect (48%) over a 16-fold range of C5 concentrations suggests that the inhibition does not result from a direct effect on the C5 molecule. The similar decay rate of purified C5 added to C5D serum and native C5 in normal serum during a 3-h incubation (Fig. 4), as well as the failure of C5D serum to inactivate C5 in a 1:1 mixture with normal serum held at 37°C for 15 min, show the absence of abnormal C5-degrading activity in C5D serum.

Thus, the data do not appear to indicate a true inhibitor of C5. Rather, we suggest that the above observations reflect the presence in C5D serum of a factor or factors which reduce(s) the sensitivity of the C5 assay. Such a factor could be present in normal serum and active at lower dilutions, but never observed in routine C5

titrations because of the very high dilutions employed (e.g., 1/160,000–1/2,560,000). For example, the inhibitory effect could be occurring at the EAC1423 step, the EAC1-6 step, or the EAC1-7 step through the action of the known inactivators of C3b (24, 25) or C6 (25), or possibly the recently proposed C7 inactivator (14). The heat lability of the inhibitory effect in these C5D sera suggests that it does not represent either the C3b or C7 inactivators, since the latter are heat stable (24, 14). A factor which interferes noncompetitively with C5 binding could also account for these findings. Efforts to characterize the responsible factor(s) and to determine whether such activity is unique to C5D serum are in progress.

The C5D homozygotes raise several points of clinical interest. In response to pyogenic infection, our C5D proband has demonstrated the ability to develop marked peripheral blood leukocytosis on several occasions, in contrast to the impairment of this response in the first reported C3D homozygote (4, 26) and apparently blunted responses in another C3D proband (26). A third C3D proband, however, is similar to our patient in the ability to sustain leukocytosis during infection (12). Thus, the contributions of the complement system to this important function remain to be fully clarified.

The fact that the proband has developed many of the classic inflammatory manifestations of SLE, i.e., cutaneous vasculitis, arthritis, glomerulonephritis, and liquefactive necrosis of the skin, in the absence of C5 (and its biologic functions) is noteworthy in itself. On the other hand, it is conceivable that C5 deficiency served to limit the severity of the renal injury so that, despite 10 yr of documented nephritis, frequently elevated levels of antibody to native and denatured DNA, and not uncommon falls in early-acting C components, the patient has maintained relatively good renal function. In this regard, the studies of Lanier and co-workers (27) on NZB/W mice are of interest. These authors hypothesized that since the nephritis in NZB mice (genetically deficient in C5) is less severe than that seen in NZB/W F<sub>1</sub> hybrids (with presumably half-normal C5 levels), the absence of C5 might have some protective role. However, when they studied the F<sub>2</sub> generation from NZB/W hybrids and correlated the frequency and degree of nephritis with C5 levels, a slightly greater incidence of nephritis in the C5D than in the heterozygous or homozygous C5-sufficient mice was found. Differences in severity were not statistically significant. The authors concluded that C5 does not contribute to the renal lesions in NZB/W mice and, in fact, may play some protective role, or that the apparent protective effect could be due to another factor genetically linked to C5 synthesis.

The important question of hypersusceptibility to infection in the two C5D homozygotes is, admittedly,

somewhat difficult to assess because of the presence of SLE and corticosteroid therapy in the proband and less complete C5 deficiency in the half-sister. The latter has had more infections than her normal or heterozygous siblings, but it can be argued that such infections are not necessarily outside the limits of normal for her socioeconomic milieu. We can state emphatically, however, that the proband stands out in our collective clinical experience as the most infection-prone SLE patient (or steroid-treated patient) we have observed. Until further examples of C5 deficiency in man are found, it seems reasonable to draw the tentative conclusion that humans with complete C5 deficiency, while not as vulnerable as C3D individuals (4, 26), may have fragile host defenses. Any added stress such as even low doses of corticosteroids or a suboptimal environment may readily expose this fragility. This is quite analogous to C5D mice which do well under routine laboratory conditions but have proven to be more vulnerable than normal mice to challenge with pneumococci (28), *Candida albicans* (29), and a mouse pathogen, *Corynebacterium kutscheri* (30). The companion paper (31) examines the capacity of C5D human serum to support several in vitro correlates of host defense and inflammation.

Much recent interest has been stimulated by the association of rheumatic disease and autoimmune phenomena with hereditary C-deficiency states, most frequently in patients with deficiency of early-acting C components (2, 3, 6-8, 10, 13). A very recently reported C7D patient, however, has several clinical features of scleroderma (9), and our C5D proband has SLE. Thus, rheumatic diseases in index cases have led to the discovery of both "early" and "late" C-component defects. Other observations have indicated a linkage of the gene controlling C2 deficiency in man with the major histocompatibility locus, HL-A (8, 32-34), and with the major locus controlling mixed lymphocyte reactions (35). Studies on this aspect in the C5D family are underway. Preliminary results indicate that C5 deficiency is not linked to the HL-A locus.\*

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